PROTHALLIA OF LYCOPODIUM IN AMERICA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 223

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(WITH TWENTY-ONE FIGURES)

Since BRUCHMANN'S great work upon the prothallia of the European species of *Lycopodium* appeared in 1898, and probably before that date, numerous attempts have been made to find prothallia in America; but, so far as the writer has been able to determine, no successful searches have been reported.

While taking a correspondence course in botany with Professor CHARLES J. CHAMBERLAIN, he suggested that I avail myself of the opportunities afforded by my location in a *Lycopodium* region and make a thorough search for prothallia. In accordance with his suggestions and directions, the work was undertaken and has resulted in the finding of 21 prothallia and over 50 sporelings, representing 5 species: *L. clavatum*, *L. complanatum*, *L. annotinum*, *L. obscurum*, and *L. lucidulum*. It is also a pleasure to acknowledge my indebtedness to Dr. W. J. G. LAND for valuable suggestions.

The first specimen, that of *L. complanatum*, was found May 22, 1916. During the same month and in September of the same year, 6 more of this species, 8 of *L. clavatum*, 3 of *L. obscurum*, 2 of *L. annotinum*, and 1 of *L. lucidulum* were dug up, making 21 in all. Although the first sporeling bearing a foot, which indicated that a prothallium was recently present, was found on April 10, 1915, it was not until May 20, 1916, that a second was found 10 miles from it. During this interval approximately 150 days were spent crawling over the ground among and around the dense beds of adult sporophytes.

At first these tedious and futile efforts would seem to indicate that prothallia are rare. Yet, when it is noted that 17 of the 21 specimens were obtained from an area not more than 10 m. square, only about one-fourth of which was actually dug up; and furthermore, that as many as 6 were yielded by one small space 4 cm.

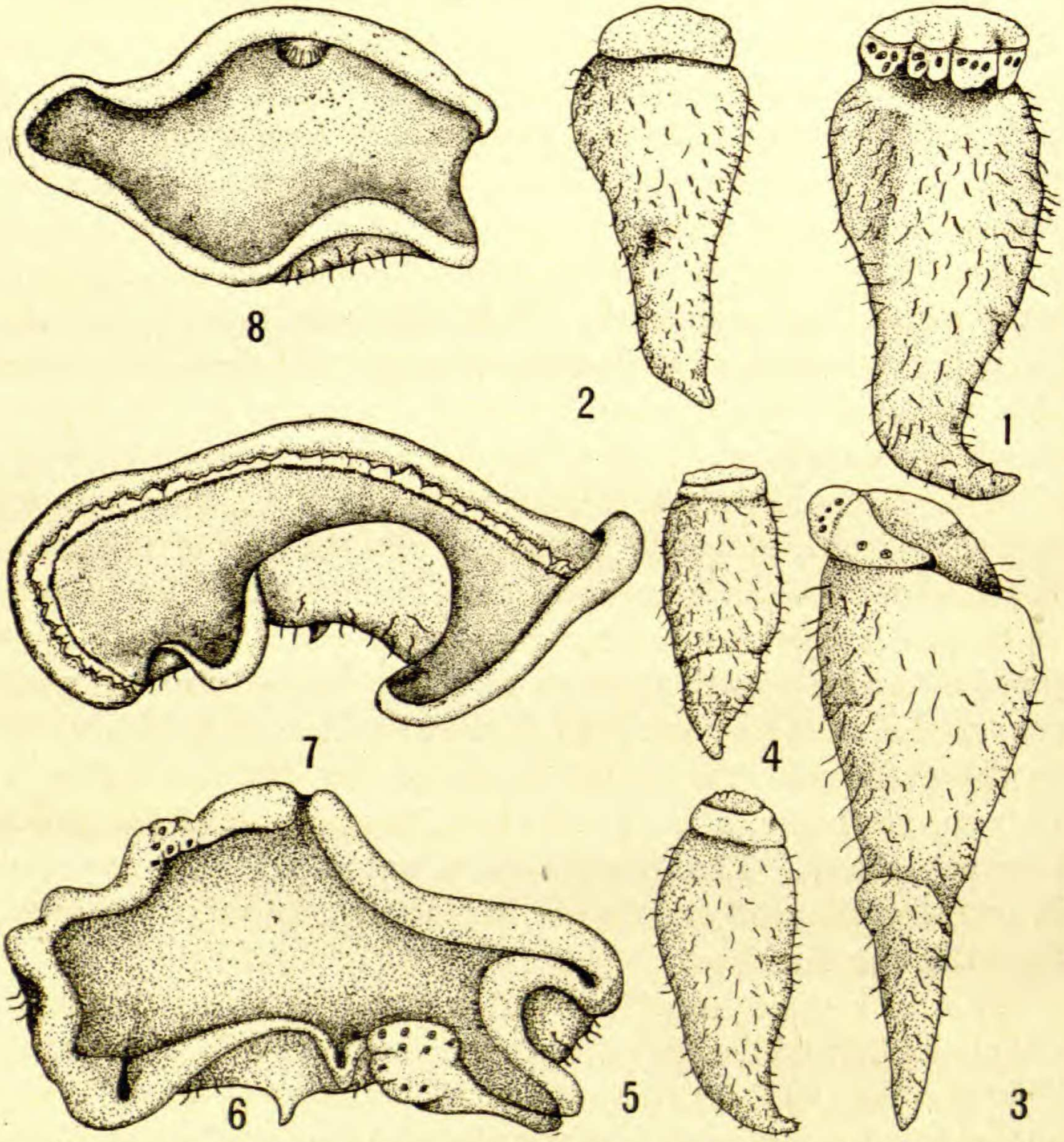
square, it seems probable that prothallia are rather abundant. The probability is even greater since the discoverer of these 21 prothallia was almost absolutely ignorant of the appearance of *Lycopodium* prothallia.

The discovery of the original locality was almost an accident. It is a place where one would least expect to find *Lycopodium* prothallia, at least those of *L. complanatum*, *L. clavatum*, and *L. annotinum*, since it is almost completely bare of adult sporophytes of these 3 species. I was wandering about looking for *Morchella*, when by chance I caught sight of an old sporeling of *L. clavatum*, growing in an exposed position between some wintergreen plants. An examination of the soil showed no prothallium. No adult sporophytes were within sight; but a sporeling is a good sign, and this encouragement led me to make the final successful search two days later.

Lying to the northwest of Marquette, Michigan, are a brewery and the remains of an old pesthouse. To the east of this pesthouse, about 700 m., is an open space bordering an open pasture on the north, a small wooded swamp on the east, and a wood composed of second growth poplar and maple on the west. A path runs to the northwest from the road which leads from Marquette to the pesthouse. Beside this path, at the distance indicated from the pesthouse, 18 of the 21 prothallia and most of the sporelings were found. The other prothallia and sporelings were found in two separate stations, the one half a mile, the other two miles from this one described.

The soil is mainly sand, covered in places by a very thin layer of humus; that within the edge of the wood is a black sandy loam. The specimens of *L. annotinum* and *L. lucidulum* shown in figs. 9 and 10 are the only ones which were found in the sandy loam where the ground is constantly shaded in summer. This specimen of *L. annotinum* is also enormously larger than the other prothallia, while that of *L. lucidulum* is decidedly the smallest. All the other specimens and most of the 50 sporelings were found in open, exposed, sandy places. The topography is uneven, rocks jutting up here and there between water-logged regions. Scattered about on the elevated regions are numerous small sandy knolls covered

partly by *Polytrichum*, and sometimes by a species of grass. In many spots these knolls are absolutely bare save for a few plants of *Polytrichum*. It was in these knolls that all but 2 of the 21 prothallia were found, as well as most of the sporelings.



FIGS. 1–8.—Fig. 1, prothallium of *L. complanatum*, first specimen found, ×5; figs. 2, 3, prothallia of *L. complanatum* (?), showing openings to the antheridia which form the lobes entirely, ×5; figs. 4, 5, prothallia of *L. obscurum* (?), crown is double, ×5; figs. 6–8, prothallia of *L. clavatum;* in fig. 6 the antheridial lobes are marked as in figs. 1 and 2; in fig. 7 the lobed edge is double; ×10.

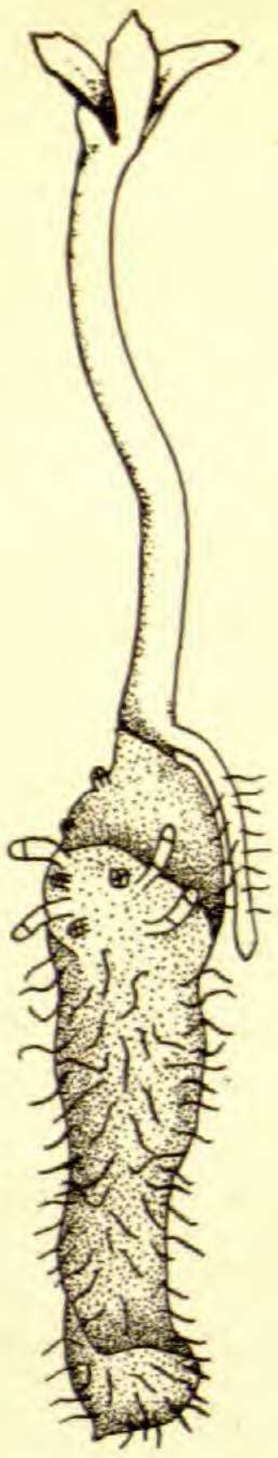
The frequent occurrence of sporelings and prothallia on these small, bare, exposed elevations suggests the idea that those spores which fall in such localities are first of all beaten into the ground

by the frequent rains; later, they are covered over by the shifting surface sand, and are finally conveyed to a favorable depth by the percolating soil water. No experiments were made to see whether this is the correct interpretation; but it was this idea which led me to investigate the knolls. This observation was heeded in all searches subsequent to the first successful one, and all yielded one or more prothallia.

So far as the elevation of the knolls is concerned, too much importance should not be placed upon it. The fact that they are elevated somewhat and exposed as they are to the air currents merely causes them to be covered by leaves less frequently than the lower or more wooded regions near them, and consequently they offer a more receptive landing to the spores which are transported thither. This idea is corroborated by the fact that many sporelings of *L. lucidulum* and *L. annotinum*, as well as 3 prothallia, were found in the middle of a trail which is frequently used by hunters. In these instances the spores were clearly trampled into the soil until they found the depth required for their growth into prothallia. I suspect that future observations may show that the prothallia of *L. annotinum* and *L. lucidulum* require more moisture for their development than the other species mentioned. The 3 specimens I found of these 2 species, although the surface indications were similar, grew in decidedly wetter places.

In searching for prothallia, soil was removed with forceps. This method was not very satisfactory, however, and 4 of the prothallia were broken or pierced by the forceps before they were seen. It would be better to remove the soil to a depth of 10 cm. and wash it through a coffee sieve. This would not only avoid the danger of damaging prothallia, but would increase the probability of finding young stages. The specimens grew at depths varying between 1 and 7 cm. The species growing nearest the surface is *L. lucidulum;* the one growing deepest is *L. obscurum*. In one hole 2 kinds, *L. clavatum* and *L. obscurum*, were seen within 2 cm. of each other.

The prothallia of *L. clavatum* and *L. annotinum* grow with the wrinkled side toward the surface, and the primary tubercle pointed downward; that of *L. lucidulum* grows erect and the sporeling



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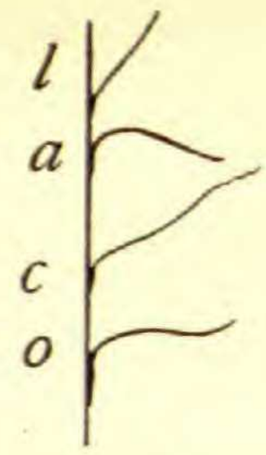

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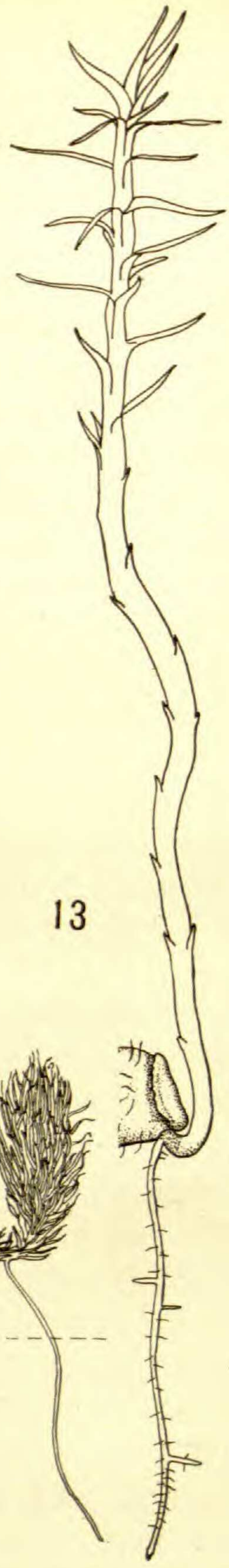

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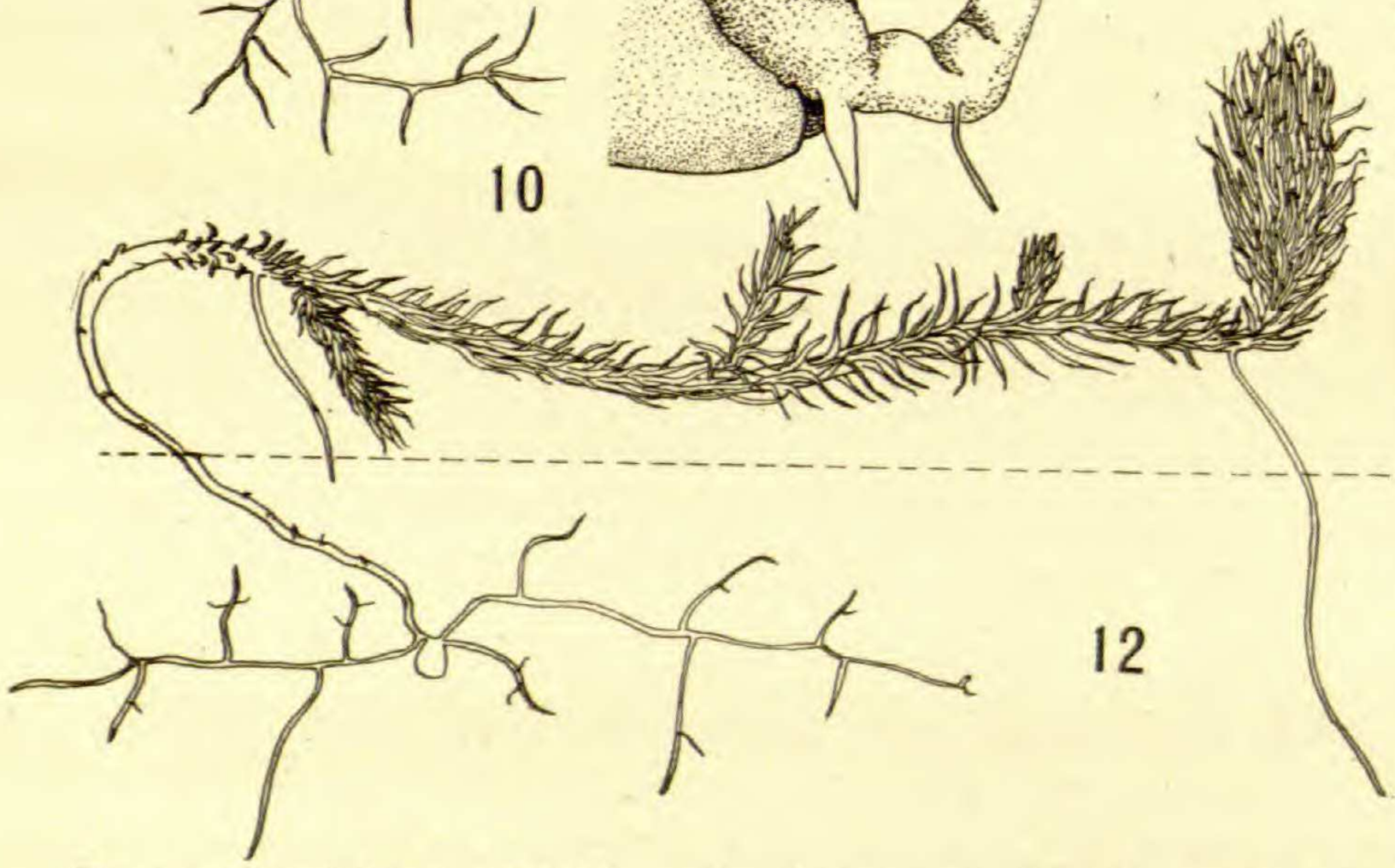

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FIGS. 9–15.—Fig. 9, prothallium bearing a sporeling of *L. lucidulum*, the only specimen found, showing 4 paraphyses, archegonia, primary tubercle, rhizoids, and an enlargement made by foot of sporeling, ×6; fig. 10, prothallium of *L. annotinum* bearing 3 sporelings, smallest one just emerging from upper tip of lower lobe of gametophyte, natural size; fig. 11, detailed drawing of largest sporeling shown in fig. 10, ×3; fig. 12, old sporeling of *L. clavatum* with foot still present, having lost erect habit of younger sporelings and assumed trailing habit of adult, ×2; fig. 13, sporeling and portion of gametophyte of *L. obscurum*, rest of gametophyte being like corresponding portions of fig. 5, ×4.25; fig. 14, diagram showing leaf contour of sporelings of *L. lucidulum* (*l*), *L. annotinum* (*a*), *L. clavatum* (*c*), and *L. obscurum* (*o*); fig. 15, diagram showing general profile of leaves of sporelings, the leaf being directed upward in *L. lucidulum* (*l*), drooping in *L. annotinum* (*a*), directed upward but bearing bristle in *L. clavatum* (*c*), bending downward then upward in *L. obscurum* (*o*), leaving the stem almost at a right angle.

arises straight out of the end lying immediately beneath the surface of the soil. The habit of growth cannot be stated definitely for *L. obscurum* and *L. complanatum*. The specimen of *L. complanatum* shown in fig. 3 was found in the same position as the specimen of *L. obscurum* shown in fig. 13. All the other specimens of *L. obscurum* and *L. complanatum* which I collected were disturbed before I saw them, and consequently their exact position was not determined, except the one in fig. 1 which grew vertically. In size, the prothallia of figs. 4 and 5, are very similar to that of fig. 13. This resemblance in size, together with a common external contour, are my only reasons for assuming that these 3 specimens belong to the same species. But here arises a difficulty. Although the prothallium in fig. 3 is about twice as large as the adult prothallium of *L. obscurum* (fig. 13) bearing a sporeling, we find the two growing in exactly the same position. This may mean two things; either the prothallia I have classed with *L. complanatum*, except no. 1, might as readily belong to *L. obscurum*, or the prothallia of *L. complanatum* grow in various positions in regard to the directions of their axes. If the axis of a prothallium of *L. complanatum* can be shown to grow always in a vertical position, and if that of *L. obscurum* can be shown to grow always in a horizontal position, the identification of the two species will then become a very simple matter if care be taken while hunting for them. I can only regret that this important point must be left for future observation to settle.[1]

Adult sporophytes of *L. clavatum* and *L. annotinum* were rare in this immediate locality, only one small patch of each having been found. A few plants of *L. complanatum* were found. *L. obscurum* and *L. lucidulum* grow in small clumps throughout the region.

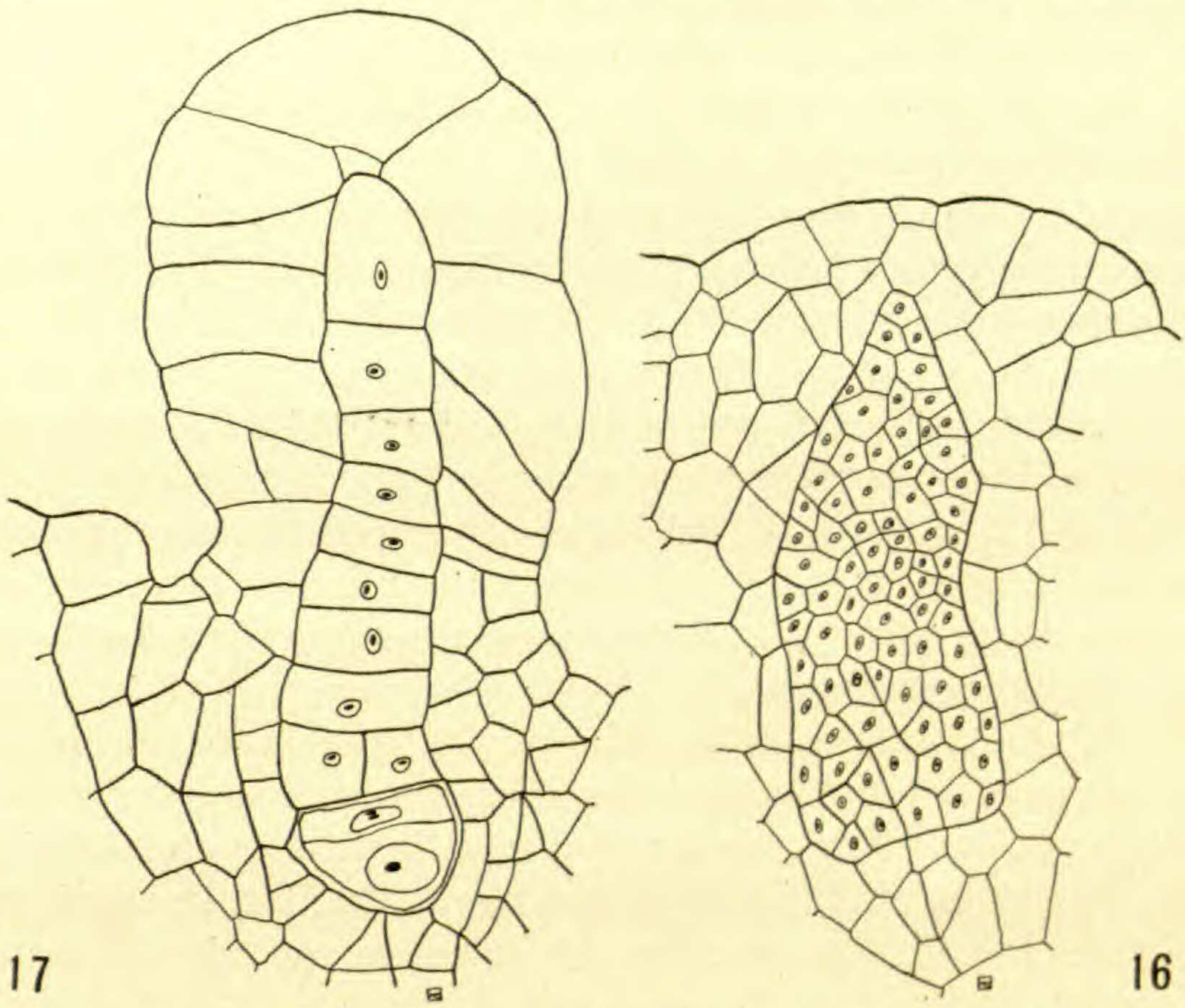
The 50 sporelings belong mainly to *L. clavatum*, but a few were found of all the other species mentioned in this paper except *L. complanatum*. Since the sporeling is the first guide in the

[1]On November 5, 1916, after this paper had gone to press, the writer found a large specimen of *L. obscurum* with sporeling attached. It was of the same color and shape as and grew horizontally like the prothallium labeled *L. complanatum* in fig. 3. This bears out the suggestion that all the prothallia classed with *L. complanatum*, except no. 1, very probably belong to *L. obscurum*.

search for prothallia as well as in their identification, I have given 2 diagrams in figs. 14 and 15 to show the specific differences between the juvenile leaves of the 4 species of sporelings which I obtained. If the descriptions of these 4 species as given in GRAY's *Manual* are followed carefully, the specific differences may readily be determined. The leaves of *L. clavatum* terminate in a bristle even in a stage as young as that sporeling of *L. annotinum* shown in figs. 10 and 11. I have not been able to separate sporelings of these 2 species at such a young stage or younger by any other character than by the presence or absence of the terminal bristle on the leaf. At such a stage the leaves have not yet assumed such very definite directions of growth as they do a little later, and as indicated in *a* and *c* of fig. 15. The sporeling of *L. obscurum* is easily identified by the 6 rows of leaves which early assume the characters peculiar to this species. Of course in the sporeling stage the leaves are fewer and more spreading than in the adult, so that a hurried examination would scarcely show the relationship between the two. I actually thought I was digging for a prothallium of *L. annotinum* while removing the soil from around the sporeling shown in fig. 13, and it was not until all the sand had been removed from among the rhizoids of the prothallium, and after the leaves had been examined under a lens, that I discovered my error. The sporeling of *L. lucidulum* cannot be confused in any way with the sporelings of any of the species I have mentioned. However, one may very readily believe that he is digging up a genuine one only to find that peculiar winged bud at the bottom of it. Yet, although I have experienced this disappointment a hundred or more times, through it I have observed that the sporelings bearing a foot grow just a trifle deeper in the ground. Generally the bud lies nearly or wholly on the surface, and unless it is rotted away, is readily seen. The sporeling of *L. lucidulum* looks exactly like one of the plants which grows from a bud, except that it has a distinct foot. Both bear leaves like an adult plant. Some of the plants in the vicinity are *Morchella*, *Polytrichum Acer*, *Populus*, *Pteris*, *Gaultheria*, *Rhus*, and *Polygala*.

The largest specimen of *L. annotinum* (fig. 10) measured 10 ×7 mm. It bore 3 sporophytes, one of which reached only 0.5

cm. above the ground, and another had just emerged from the prothallium. In color it resembled closely the gametophyte of *Botrychium virginianum*. The specimens of *L. clavatum* were much smaller and almost white in color. They varied in size from 6.5×5 mm. to 5×3 mm. The largest fragment measured 7×5 mm., and the smallest 2×1 mm. Figs. 6, 7, and 8 show the entire specimens. The fragments were more twisted and wrinkled than



FIGS. 16, 17.—Fig. 16, antheridium of *L. clavatum* in sperm mother cell stage, ×365; fig. 17, archegonium of *L. complanatum* immediately before neck canal cells break down, the lowest neck canal cell being double, ×365.

these, but both the fragments and the entire specimens showed a distinct crown on the border, which in one case (fig. 7) was double, the inner crown having an irregular outline. Each of the entire prothallia showed the primary tubercle very distinctly, near the middle, on the ventral side.

The prothallia of *L. complanatum* were all entire except one which had the lower portion broken off. Seven of these prothallia,

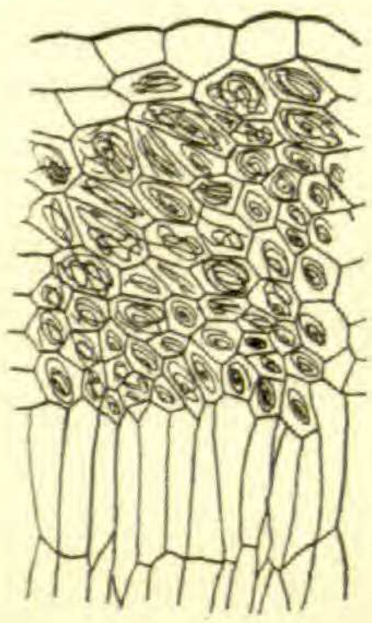
illustrated by figs. 1, 2, and 3, bore a single crown which was more or less lobed. In section (fig. 19) these lobes proved to be masses of antheridia. The crown on each of the 3 prothallia of *L. obscurum* (figs. 4, 5) was double. An accident to the sections prevented a study of the relation between the lobes and the location of the sex organs in these prothallia. The crown was unequal in every specimen of *L. complanatum* and *L. obscurum*, and the lobes appeared only at one side, as shown in fig. 3.

The one prothallium of my collection which may cause some readers to question is that of *L. lucidulum*. I have found 10 sporelings and only one prothallium, but this single prothallium fortunately has a sporeling attached. This fact alone would not serve to convince a botanist in doubt of its genuineness. However, there are 5 reasons why I am convinced of its nature. Fig. 9 shows these 5 points. They are (1) archegonia, (2) paraphyses, (3) rhizoids, (4) a primary tubercle, and (5) the sporeling arises from it like the sporeling of any recognized gametophyte; there is a foot, and a primary root originates at the point where the stem breaks through. Certainly all these features cannot be connected with a young plant of *L. lucidulum* of asexual origin or with an associated fungus growth.

This prothallium of *L. lucidulum*, which, like that of *L. obscurum*, I believe is new to botanists, shows some very interesting evolutionary points. The body is somewhat cylindrical, but not entirely so. It is somewhat more flattened longitudinally than the figure shows. In this figure the flat side is turned toward the reader. Near the middle it makes a short twist toward the left. In general outline it looks like a prothallium of *L. complanatum* in the making, but on the upper lobed region are 4 paraphyses. Such a feature suggests a transition stage between the *Phlegmaria* type of prothallium, as represented by *L. Selago*, and such a form as *L. complanatum*. The specimen contained no chlorophyll so far as I was able to determine.

Among the 50 sporelings gathered there was a variation in age between 1 and 5 years. Each season's growth above the soil could be determined definitely by the alternate regions of longer and shorter leaves on the species *L. clavatum* and *L. annotinum*.

Fig. 11 shows in detail the largest sporeling of *L. annotinum* growing from the prothallium represented in fig. 10. The sporeling of *L. clavatum* shown in Fig. 12 was one of the largest, and serves to indicate the size one may obtain still bearing the foot. It is very possible, even for one who has studied carefully the figures of *Lycopodium* prothallia given in the various papers upon the subject, to mistake other forms of tubers for them. The sex organs may be indistinct or undeveloped, so that identification by means of



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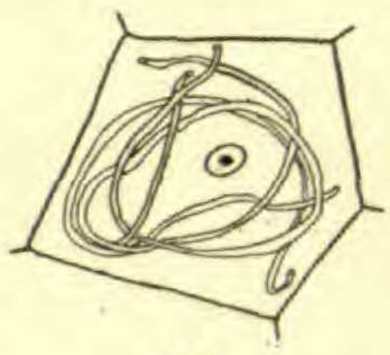

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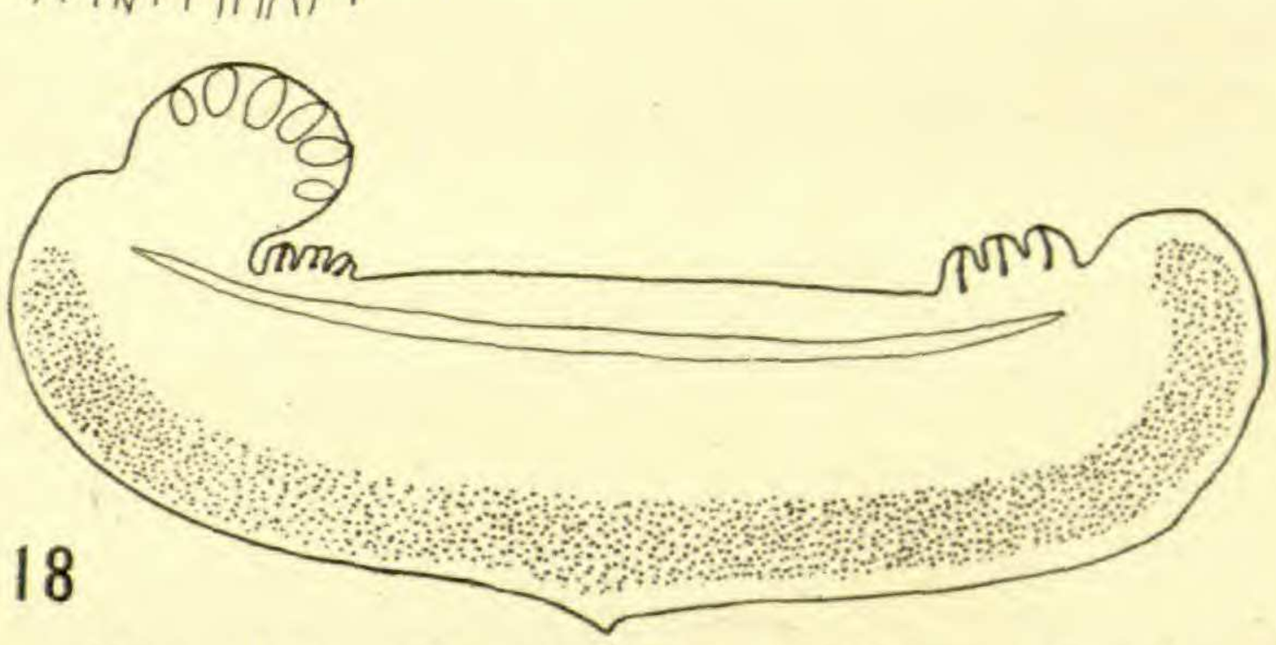

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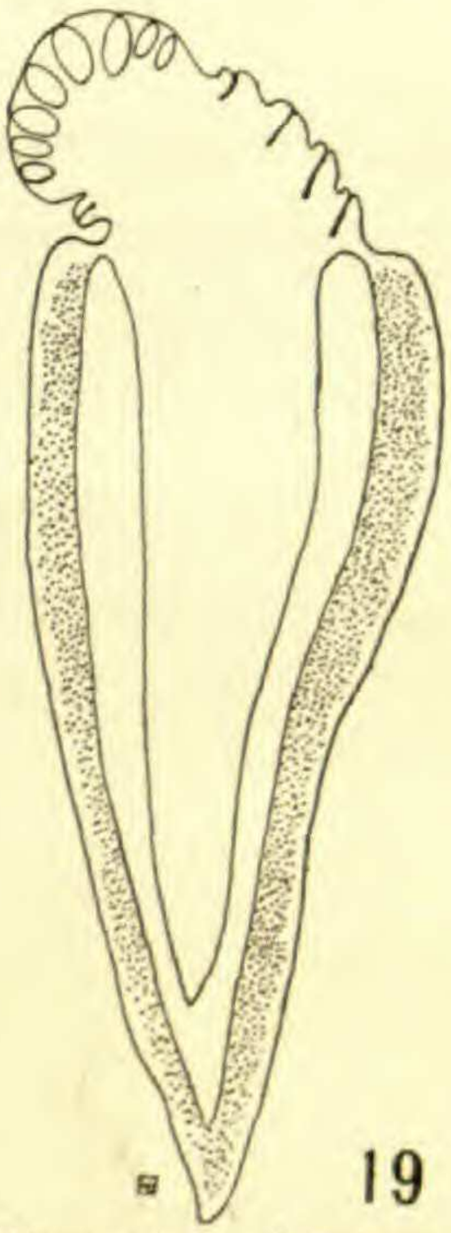

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FIGS. 18–21. Fig. 18, diagram of tissue regions in prothallium of *L. clavatum* (antheridia and archegonia indicated); fig. 19, diagram of median region of prothallium of *L. complanatum*, the fungus-infected region being indicated in this and fig. 18 by dotted shading; fig. 20, detailed sketch of fungus-infected region, showing its location beneath the epidermal tissue, ×75; fig. 21, single cell with endophytic fungus coiled within (fungus passes freely from one cell to another by piercing the cell wall), ×750.

them is impossible in such an instance. The number and variety of small tubers which grow in the soil of a wood are both large and confusing. Some of them are surprisingly similar in all outward appearances to small prothallia of *L. complanatum*. I do not doubt that I threw away some genuine specimens because they appeared to be tubers. I know that I retained some tubers and even sectioned them in paraffine because I thought they were prothallia.

To establish definitely whether the forms that I have collected are genuine prothallia of *Lycopodium*, therefore, I have drawn figs. 16, 17, 20, and 21 to show an antheridium, an archegonium in which the lowest of the neck canal cells is double, and 2 detailed sketches of the fungus-infected region. The diagrams in figs. 18 and 19 indicate the tissue regions shown by sections of 12 prothallia.

This article seeks to establish the fact that the prothallia of *Lycopodium* have been found in America; to make known to those botanists who may later desire to find prothallia for themselves, what the conditions were under which the specimens collected were found; and to announce the discovery of 2 new species of *Lycopodium* prothallia, namely, *L. obscurum* and *L. lucidulum*. Concerning the development and structure of the American *Lycopodium* gametophytes, the writer hopes to deal in a later paper.

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